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WIREWORM DISTRIBUTION IN EAST ANGLIA

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DURING the years 1944–54, 1,289 grass fields in East Anglia were sampled for wireworms, to give advice on cropping after ploughing (Anon, 1944). This paper is an attempt to extract information from the available records of the survey, which was not random but based solely on advisory requests received up to October 1946 by the Advisory Entomology Department of the School of Agriculture, Cambridge, and since then by the Eastern Province of the National Agricultural Advisory Service. Only old grass, or fields newly-ploughed from old grass, have been included in the results described here. The exact age of the grass fields was not always known, but it is unlikely that there would be any which were less than ten years old. Towards the end of this period fewer fields were being ploughed up and chemical control of wireworms was widely practised, so that requests for sampling declined.

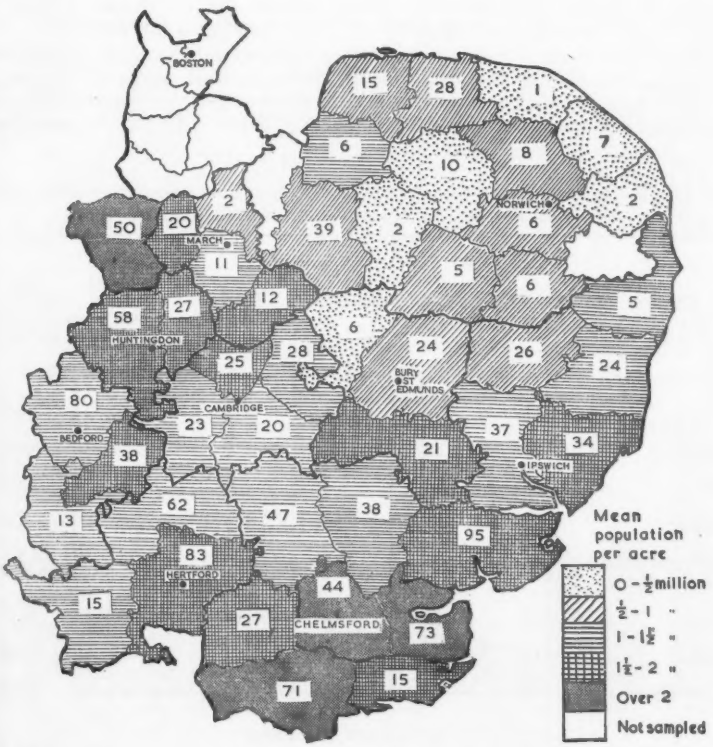

Fig. 1. Wireworm populations of grass fields in advisory districts, 1944–54. Inset figures indicate number of fields sampled.

METHODS

Samples, usually twenty in number, were taken from each field with a 4-inch diameter soil corer (Anon, 1944), either before or after ploughing. The depth of each sample was normally six inches in unploughed grassland, and where already ploughed to just below the depth of ploughing—usually about nine inches. The wireworms were extracted from the soil by the flotation process, which has been fully described by Cockbill, Henderson, Ross and Stapley (1945). Most of the sampling was done during the autumn and winter, and the sampling year started in September.

The predominant genus was *Agriotes;* other genera were recorded when found but as the numbers of these were usually very low, they are not included in the totals. The wireworm counts given are the total population of all sizes, though advice was based only on those 5 mm long and over. In this account the wireworm populations have been grouped into the following categories; 0–500,000; 501,000–1,000,000; 1,001,000–1,500,000; 1,501,000–2,000,000 and over two million.

DISTRIBUTION OF SAMPLING

Requests for wireworm counts were usually made by farmers to the district advisory officer, who sent them on to provincial headquarters. The interest of the farmer and the district advisory officer therefore influenced the distribution of counts, and there was also a natural and increasing tendency for more requests to come in from areas of higher wireworm populations.

The pre-war type of farming, which also influenced the acreage of grass being ploughed, was another important factor. As the area of grassland ploughed up yearly in each county was not available, the pre-war acreage of permanent grass, represented by the figures for 1939, is given in Table 1. This gives some indica-

TABLE 1

No. of Fields Sampled in Counties, Their Mean Wireworm Population over a 10-year Period and Acres of Permanent Grass in 1939

County	1944–47	1947–50	1950–54	Total	Acres of Permanent Grass in 1939 (*to nearest 1,000*)	Mean Wireworm Population (*in 1,000s*)
Essex	117	228	65	410	303	1,647
Suffolk ..	23	84	70	177	213	1,108
Herts	58	80	22	160	136	1,473
Norfolk ..	27	78	30	135	268	776
Hunts and Soke of Peterboro'	45	53	37	135	102	1,782
Beds	50	38	43	131	121	1,275
Cambs ..	17	46	34	97	63	1,366
Isle of Ely ..	9	12	23	44	40	1,577
Total ..	346	619	324	1,289	1,246	

tion of the grassland available for ploughing. The number of fields sampled, grouped for convenience into three- or four-year periods, and the mean wireworm population for each county are also given in this Table. The three periods 1944–47, 1947–50 and 1950–54 were chosen because a preliminary inspection of the figures suggested that, within these periods, the mean wireworm populations were fairly uniform.

The sampling distribution corresponds generally with the amount of permanent grass, except for Norfolk where the lower mean wireworm population density probably accounts for the fewer samples taken. Histograms of wireworm categories for each county are shown in Fig. 2.

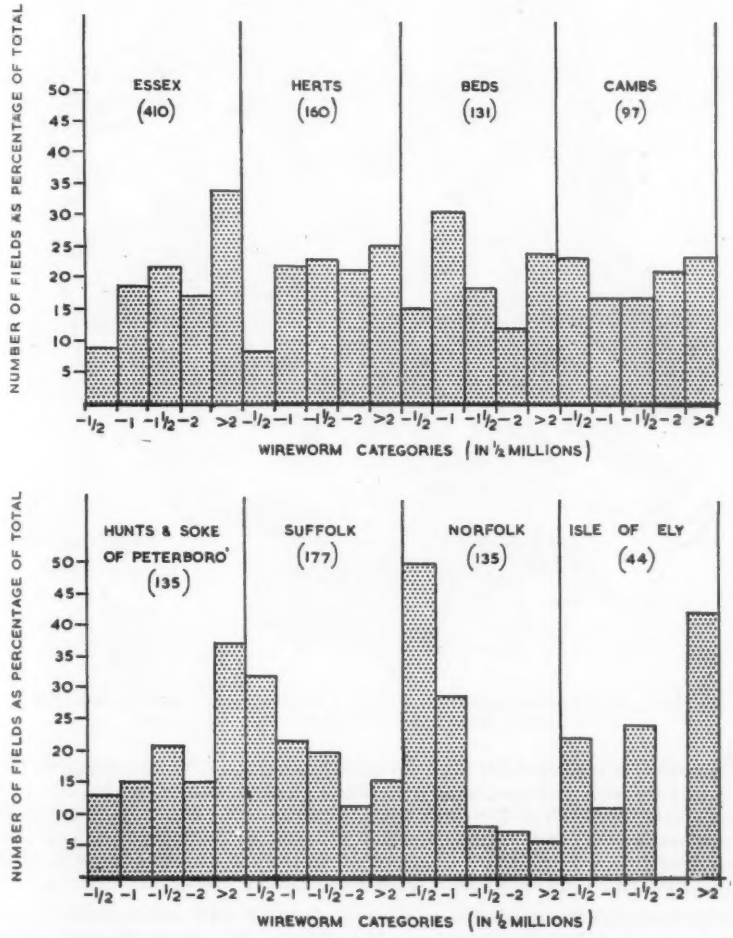

Fig. 2. The distribution of wireworms according to size category for each county in the period 1944-54. Numbers of fields sampled are given in brackets.

For the study of geographical trends, the distribution of wireworm density should be correlated with ecologically similar areas. As this was impracticable, and as counties are inconveniently large and diverse units, the mean densities were mapped according to N.A.A.S. districts, of which there are several per county (Fig. 1). A map of soil types (Fig. 3) is given for comparison. Close correlation is not to be expected, but there is an indication that counts are lower on the Breckland and the lighter soils of Norfolk generally. Populations tend to be higher on the medium to heavy soils such as the London clay and Brickearths of Essex, and the heavy clays of Hertfordshire, Huntingdonshire (including the Soke of Peterborough) and parts of Suffolk.

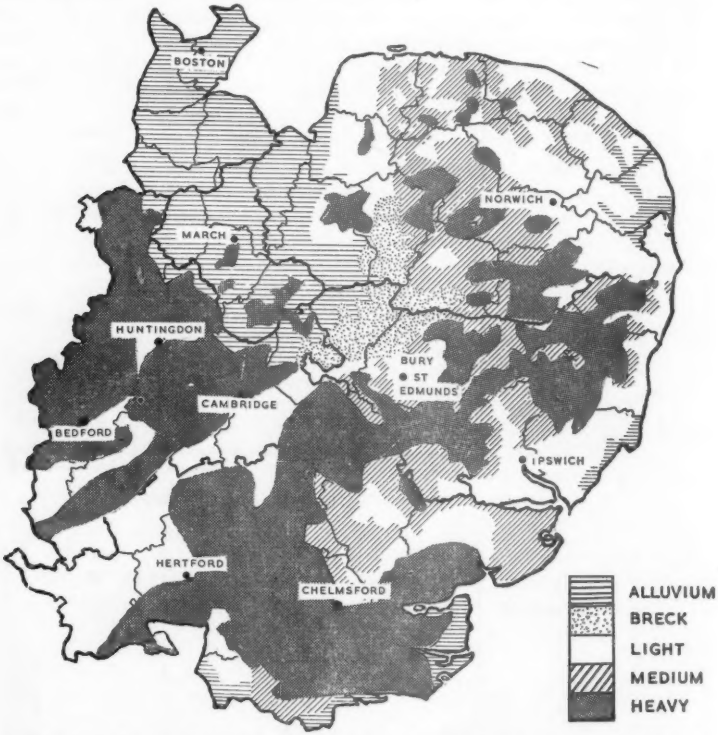

Fig. 3. Soil types in the Eastern Region. Adapted from a map by the Farm Economics Branch, School of Agriculture, Cambridge.

Records for the 619 fields sampled in the period 1947–50 were examined for a relationship with soil type. This period was chosen as the average counts were fairly stable for each of the three years. Unfortunately the only records of soil types available were in the rather general terms of light, medium and heavy. The results shown in Table 2 clearly confirm the association of high wireworm population and heavy land previously noted (Anon, 1944).

Fig. 4 records the mean counts for each year for all counties and separately for Essex which had the largest number of fields. The standard errors of these yearly means were calculated and are shown in broken lines on the graph. As fields are not selected at random it is doubtful if much reliance should be placed

TABLE 2

Correlation of Wireworm Populations and Soil Types, 1947–50

Soil Type	No. of Fields	Mean Population *in thousands*
Light	114	712
Medium	443	1,480
Heavy	62	2,588

on the smaller fluctuations even though they may represent significant differences. Nevertheless, it seems almost certain that the general downward trend is a real one.

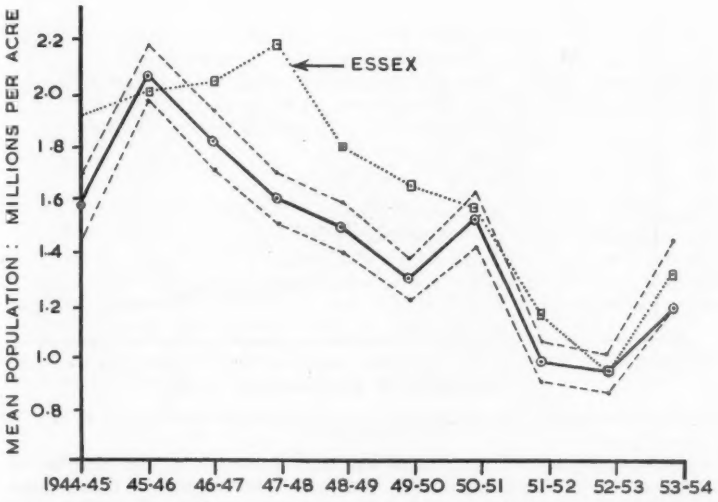

Fig. 4. Annual mean wireworm count in the Eastern Region, 1944-54. The broken lines show the standard error of the mean. The dotted line shows the mean yearly count in Essex.

Although some general decline in total wireworm population may be expected because of the gradual reduction of permanent grassland, and later to the fairly general use of soil insecticides, these factors are unlikely to have greatly affected the population in permanent grass fields. It is possible that there was a tendency for grass fields sampled in the earlier years to be older, on the average, than those of later years, but it is not possible to assess the importance of this.

If the decline was real, it was thought that some light might be thrown on its causes by studying the distribution of the various sized groups of wireworms in the different years. The figures for Essex for the periods 1944–48, 1948–51 and 1951–54 were examined and the results are shown in Fig. 5.

It can be seen from Fig. 5 that a very high proportion (nearly 75 per cent) of the total number of wireworms is under 10 mm in length and 35 per cent under 5 mm. Wireworms of 20 mm and over are grouped together, which accounts for the increase in number in this category as some reach a length of 25 mm. This distribution is typical of those recorded by Salt and Hollick (1944) and Ross, Stapley and Cockbill (1947).

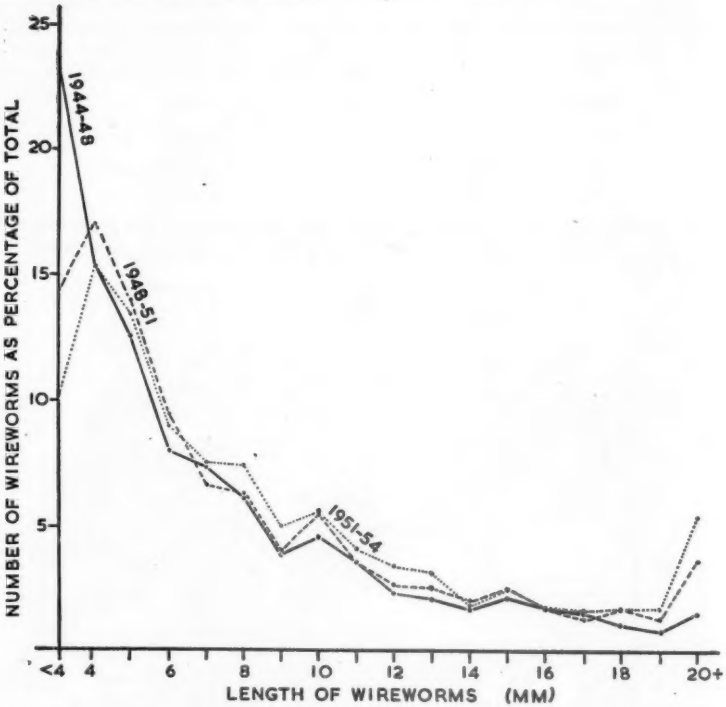

Fig. 5. The wireworm size distribution for Essex, for the periods 1944–48, 1948–51 and 1951–54.

With the exception of wireworms less than 4 mm long the graphs for the three periods are remarkably similar and there is no suggestion that the composition of the population has varied. The greater number of small wireworms recorded in 1944–48 is almost entirely due to the fact that in 1946 weather conditions made it possible to sample forty fields in July and August, at a time when very small newly-hatched wireworms were present. In the other two periods few or no fields were sampled in these months.

Ross, Stapley and Cockbill (1947) have suggested that wireworm counts show certain seasonal trends throughout the year, and the results for Essex were scrutinized from this point of view.

The mean monthly counts for all years are shown in Table 3. The figures for September to May, inclusive, are fairly uniform, with an indication that the counts are slightly higher from December to May, inclusive. It is difficult to draw any conclusions from the June to August results owing to the small number of fields sampled, especially in June. The figure for July seems high, but this is due to the July, 1946 count already mentioned. As this was a wet month, there

TABLE 3

Mean Wireworm Count per Month—Essex 1944–54

Month	No. of Fields Sampled	Mean Population per Acre (*1,000s*)
September ..	45	1,665
October	51	1,714
November ..	54	1,584
December ..	26	2,333
January	43	1,953
February ..	65	2,021
March	53	1,795
April	12	1,968
May	8	1,951
June	2	1,000
July	29	2,298*
August	22	1,426

*1,200, excluding 1946.

was possibly a greater survival than usual of newly hatched larvae. If one disregards 1946, the July average is reduced to around 1,200,000 and the general picture agrees with that of Ross, Stapley and Cockbill (1947), who found that the maximum counts were generally recorded during the period December to April and minimum counts during June, July and August.

SUMMARY

In East Anglia, 1,289 old grass fields were sampled for wireworms, to give advice on cropping in the period 1944–54. Distribution of wireworm population density is shown by a map. The population density shows a general downward trend during the period, but an examination of size categories of wireworms did not throw any light on the reasons for the trend. Maximum counts were recorded during the months December to May, inclusive.

I am greatly indebted to Dr. H. C. Gough who suggested this analysis, and for his help in the preparation of the script.

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CONTROL OF *GLOEOSPORIUM PERENNANS* WITH FORMULATIONS OF ERADICANT FUNGICIDES

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ORCHARD measures for controlling *Gloeosporium* rot of stored apples (bitter rot), normally include the excision of all tree infections of the causal organisms, *G. perennans* Zeller and Childs and *G. album* Osterw., during late winter pruning. However, since sources of the disease range from branch cankers and dieback lesions to mummified fruitlets and inconspicuous saprophytic infections on stubs, snags and fruiting spurs, measures involving excision are laborious and only partially effective. A more practical approach seems to lie in the application of eradicant fungicide sprays which have been successfully used, in the dormant season, for the inhibition of sporulation of other pathogenic fungi.

Wilson (1942, 1950) found that winter applications of arsenite and sodium pentachlorophenate sprays reduced sporodochial development on twig infections of *Sclerotinia laxa* on stone fruit trees. Byrde (1952), in exploratory work with eradicant fungicides, showed that phenylmercuric chloride, at 0·2 or 0·3 per cent gave a 90 per cent reduction in sporulation of *S. laxa* and *S. fructigena* on mummified apple and plum fruits which had overwintered on the tree. Byrde, Crowdy and Roach (1952), also found that sodium pentachlorophenate and phenylmercuric chloride checked the sporulation of *Nectria galligena* on established stem cankers on apple. Subsequently Taylor and Byrde (1954) showed that an eradicant fungicide containing 0·3 per cent phenylmercuric chloride, applied in mid March to trees infected with apple canker, effected a marked reduction in the incidence of *Nectria* eye-rot on the apple fruits at harvest. Recently, Eaves, Hockey and Ross (1958) have obtained control of apple storage rots, due to *G. album* and *Penicillium* sp., with phenylmercuric acetate sprays. They suggest that this control may have been effected by a reduction in the amount of inoculum in the plots, since no sprays were applied after the fruitlet stage.

The present study is an examination of the possible value of late winter sprays of eradicant fungicides for the orchard control of *G. perennans*. Preliminary spray trials showed that a 0·3 per cent aqueous suspension of phenylmercuric chloride and aqueous solutions containing 0·4 per cent sodium dinitro-*ortho*-cresolate and 0·6 per cent sodium pentachlorophenate were ineffective in suppressing conidial production or checking mycelial spread in artificially induced cankers of *G. perennans*; it was apparent that a more tenacious formulation of the fungicides was required. Somers (1956), in examining possible additives to copper fungicide sprays, showed that polyvinyl acetate was the most effective in increasing the tenacity of a spray deposit without inhibiting its biological activity. It was therefore decided to carry out pilot field trials in 1956–57 with this synthetic latex as a sticker for eradicant fungicides, both in paint and spray applications on cankers. Linseed oil was also used with the sprays because of its known ability to give high initial deposits and tenacious residues (Somers, 1956).

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* Now at Lenton Experimental Station, Nottingham.

FUNGICIDE FORMULATIONS

A. Sodium pentachlorophenate (PCPNa) paint, solid at 0·6 per cent dispersed in 90 per cent (v/v) polyvinyl acetate latex (PVA). (The latex was thinned with water to give improved spreading.)

B. Phenylmercuric chloride (PMC) paint, solid at 0·3 per cent dispersed in 90 per cent (v/v) PVA.

C. 0·3 per cent Phenylmercuric chloride in aqueous suspension.

D. 0·3 per cent Phenylmercuric chloride in 5 per cent (v/v) PVA.

E. 0·3 per cent Phenylmercuric chloride mixed with linseed oil emulsion (at a final concentration of 2 per cent) and made up with water to requisite volume. The linseed oil was emulsified with texofor F20 (a polyethylenoxy non-ionic surface-active agent), at a final concentration of 0·001 per cent, to give a quick-breaking emulsion that agglomerated the phenylmercuric chloride.

F. 90 per cent (v/v) PVA paint without added fungicide.

All formulations containing phenylmercuric chloride were prepared by wet grinding the pure material with an equal weight of kaolin and 1·4 per cent methyl ethyl cellulose (at a final concentration of 0·007 per cent). The chemicals were all of laboratory grade and the concentrations were on a w/v basis, unless otherwise noted. The PVA latex had 60 per cent solid content and less than 0·3 per cent free monomer. The paints A, B and F were applied by brush and the suspensions C, D and E were sprayed with a hand atomizer.

EXPERIMENTS IN 1956

The paint formulations (A and B) and a PVA paint without added fungicide (F) were tested against cankers of *G. perennans*; an untreated control (O) was also included. A row of 32 ten-year-old dwarf pyramid Cox trees (4 feet apart) was divided into eight randomized blocks, each containing one tree for each of the four treatments. Each tree carried three cankers which had grown to 11 cm in length by the spring of 1956; the cankers had been artificially induced, in the autumn of 1955, by placing a small piece (0·5 × 0·5 cm) of malt extract agar, bearing mycelium of *G. perennans*, over a pin stab wound in a straight length of branch, and protected with self-adhesive crepe rubber for twenty days. The paints were applied on July 6 and the output of conidia, from acervuli produced on the surface of the cankers, was recorded at eight sampling dates between July 25 and November 14. Spore samples were collected from the cankers by immersing them for 15 minutes in water contained by a polythene sleeve (Sharples, 1958*a*); the washings from each canker were centrifuged and the average concentration of spores determined from at least four haemocytometer counts. The overall average reduction in spore output, from the eight samplings, for each treatment is given in Table 1. To achieve constant variance in the

TABLE 1

Effect of Fungicide Paints on the Sporulation of Cankers of G. Perennans

	Mean Spore Production (×*10,000*)				Level of Significance		
	B *PMC*	A *PCPNa*	F *PVA*	O *Control*	*5 per cent*	*1 per cent*	*0·1 per cent*
Overall mean..	1·02	2·36	5·78	8·83	F<O	B<A	A, B<F, O
Percentage of control ..	11·6	26·7	65·5	—			

Paints applied July 6, 1956. Cankers sampled July 25–November 14, 1956.

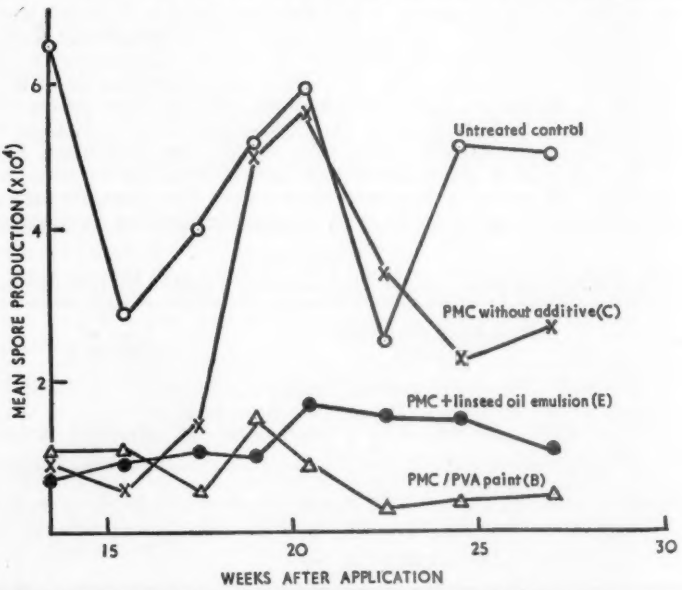
analysis of the data, the recorded values were transformed into logarithms. Thus the mean spore production, given below, is the antilogarithm of the average of the logarithmic values.

Table 1 shows that both fungicide paints suppressed sporulation by cankers of *G. perennans*, and that phenylmercuric chloride was more effective than sodium pentachlorophenate. This effect continued for at least four months, when the overall average reduction of more than 88 per cent was still being maintained by the mercury paint. Spore production from cankers treated with PVA not containing a fungicide (F), was restricted for only three weeks. Thereafter the effect diminished rapidly, and subsequent spore production from these cankers was very erratic. The spores produced by cankers treated with the paints A and B proved to be viable.

EXPERIMENTS IN 1957

In 1957, the phenylmercuric chloride formulations C, D, and E were tested as late winter eradicant sprays against wood infections of *G. perennans*. Their effectiveness was compared with that of the phenylmercuric chloride paint (B); untreated controls (O) were also included.

A row of twenty-five Cox trees, similar to those used in the previous trial, was divided into five randomized blocks, in which each tree (one unit plot) carried five cankers which had been artificially induced by pin stab mycelial inoculations in the autumn of 1956. The treatments were applied on March 11, 1957, and spore production was recorded by a series of fortnightly samples between June 12 and September 13, covering the period of fruit development on the trees. The overall average reduction in spore output effected by the various formulations of phenylmercuric chloride is given in Table 2. These results were obtained from eight samplings, and the individual mean values for the treatments B, C, E, and O are plotted in the Figure. Table 2 also includes analyses



Effect of PMC formulations on the sporulation of cankers of *G. perennans*.

of the mercury residues (given as μg phenylmercuric chloride) of three cankers of each treatment collected towards the conclusion of the experiment on October 4, when formulation E was the only treatment still inhibiting sporulation, (Sharples, 1958*b*). Mercury was determined by the method of Abbott and Johnson (1957).

TABLE 2

Effect of Different Formulations of Phenylmercuric Chloride on the Sporulation of Cankers of G. Perennans

	Mean Spore Production (× *10,000*)					Level of Significance	
	C *Without Additive*	D *Diluted Paint*	E *Linseed Oil*	B *Paint*	O *Untreated Control*	*1 per cent*	*0·1 per cent*
Overall mean	2·39	2·10	1·19	0·76	4·51	E<D	B, C, D, E<O B, E<C B<D
Percentage of control ..	53·0	46·7	26·4	16·9	—		
Residue μg PMC/g canker	1·7	10·0	7·1	19·3			

Formulations applied March 11, 1957. Cankers sampled June 12–September 13, 1957.

The sprays of phenylmercuric chloride without additive (C), and with 5 per cent PVA (D) reduced canker sporulation, as compared with the control, only until mid July, seventeen weeks after application. The PVA paint formulation (B) was again consistently effective in reducing spore output, and gave an overall average reduction of over 80 per cent until mid September, about six months after application. The linseed oil spray (E) was not significantly different in efficiency from the paint (B), and maintained an overall average reduction of 75 per cent in the production of viable spores during the period of fruit development. Both treatments B and E were significantly more effective (at the 0·1 per cent level) than C, the spray of phenylmercuric chloride without additive. Although none of the treatments inhibited the spread of fungus within the host tissue, there was no evidence of any of the formulations retarding the formation of healing callus around the lesions.

Further data (Sharples, 1958*b*) show that the paint formulation (B) applied in March 1957 inhibited canker sporulation for six months, whereas the same formulation applied in the July 1956 experiment was effective for at least twelve months. Thus, the durability of eradicant materials applied to cankers will probably vary considerably according to the time of application and the weather conditions during the treatment.

The chemical analyses show that the additives, linseed oil and PVA, markedly increased the residue level of the phenylmercuric chloride sprays, either through increasing the initial deposit and/or the tenacity of the phenylmercuric chloride suspension. However, the reduction in canker sporulation cannot be directly related to the increased residue level, which suggests that the PVA latex is reducing, to a certain extent, the fungitoxicity of the phenylmercuric chloride deposited on the canker.

Recent work by Sharples (1958*b*) has emphasized that full control of the fruit rot diseases *G. perennans* and *G. album* requires thorough eradication of existing tree infections, carried out over several successive seasons, and the prevention of the development of fresh lesions. There are obvious advantages in replacing pruning techniques by fungicide spray treatments, and the present work has shown that suitable formulations can markedly reduce the sporulation of fungal infections. The application of a PVA fungicide paint, although effective biologically, would normally prove too laborious to be of value, except perhaps in nurseries and young plantings where excision of cankers is likely to interfere with the build-up of the main framework of young trees. Paint treatment of small stub and snag infections is also clearly impracticable and, for these cases in particular, it would be necessary to apply an eradicant fungicide as a spray.

The best spray formulation tested was that incorporating a quick-breaking linseed oil emulsion which flocculated the phenylmercuric chloride particles, giving them greater tenacity, and probably increased initial deposit (Somers, 1956), than phenylmercuric chloride without additive. However, Sharples (1958*b*) has shown that, at a concentration effective in reducing canker sporulation, phenylmercuric chloride does not arrest the subsequent growth of established lesions, although spore production from *Gloeosporium* cankers falls off rapidly after the first year. In addition, spray formulations containing 0·3 per cent phenylmercuric chloride would be very expensive if used in established orchards and might also prove hazardous to the operator. Thus, at the moment, although no commercial formulation can be proposed it is suggested that linseed oil should provide a useful basis for future trials of eradicant fungicides.

SUMMARY

In pilot scale trials of eradicant fungicides, 0·3 per cent phenylmercuric chloride, formulated as a paint in polyvinyl acetate latex, effected an 80 per cent reduction in spore output from stem cankers of *Gloeosporium perennans* for at least six months. A spray formulation of phenylmercuric chloride in 2 per cent linseed oil emulsion proved to be almost as effective as the paint, and reduced sporulation by 75 per cent during the period of fruit development. The additives, polyvinyl acetate latex and linseed oil, both markedly increased the residue level of the phenylmercuric choride sprays.

We are indebted to Mr. R. W. Marsh and Dr. R. J. W. Byrde for their advice and interest in this work, to Mr. J. A. Pickard for the mercury analyses, and to Mr. G. M. Clarke and Miss M. E. Holgate for analysis of the experimental data. Valuable experimental assistance was given by Miss N. M. Waugh.

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FUNGICIDAL CONTROL OF CLUB ROOT

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IN a trial carried out in 1956 PCNB, when used as a root dip at transplanting, gave a good control of club root, but this was not correlated with increased yield because of possible phytoxicity in the rhizosphere (Rosser, 1957). A trial was, therefore, carried out in 1957, on heavily infected land at Wolverhampton, when the fungicide was applied as a dust before planting out. Healthy cabbage plants were planted on June 26 in a randomized block layout in which there were eight blocks, each of six plots. Each plot comprised two outer rows of six plants each and a central row of five plants. The rows were 24 in. apart, the plants 18 in. apart and a 36 in. wide path surrounded each plot.

There were five fungicidal treatments and an untreated control. Pentachloronitrobenzene dust was applied at rates equivalent to 30 lb per acre, and 60 lb per acre; and tetrachloronitrobenzene dust was applied at a rate equivalent to 8 lb per acre. These dusts were carefully broadcast on the appropriate plots thirteen days before planting, and the soil surface was then lightly forked and thoroughly raked to ensure incorporation of the fungicide in the top 4 in. of soil.

TABLE 1

Effect of Treatments on the Crop. (*Means per Plot of 17 Cabbage Plants*)

Treatment	No. of Healthy Plants	No. of Marketable Heads	Weight of Tops *lb*	Weight of Roots *lb*
PCNB .. .. .. 30 lb per acre	3·13	8·5	22·75	4·96
PCNB .. .. .. 60 lb per acre	2·63	8·25	25·75	4·93
TCNB .. .. .. 8 lb per acre	2·38	6·75	19·75	5·26
Mercuric chloride .. 1 in 2,000	6·63	14·37	40·75	4·61
Control	1·88	8·38	24·38	5·54
S.E. per plot	1·89	3·65	10·63	0·62
Significant difference $p = 0·05$	2·54	3·74	8·74	0·63
$p = 0·01$	2·73	5·04	11·80	0·85

At planting-out the remaining two treatments were applied at the rate of half a pint of a 1 in 2,000 solution of mercuric chloride, and a similar amount of a 1 in 320 solution of PCNB, to the appropriate dibble holes. Because of the extremely dry soil conditions, where no liquid treatment was involved, one half pint of water was applied to each plant, including the controls, after planting out.

(75345) A** 2

Very shortly after the trial was begun the drought conditions were broken. The monthly rainfall figures for July, August and September were 3·17, 3·84 and 4·27 inches, compared with the respective mean averages of 2·26, 2·42 and 1·81 inches. No check to growth was noted in any treatment, except where the liquid formulation of PCNB was used. This proved to be lethal, and on July 31 the twenty-eight surviving plants were all severely stunted and wilting. These plants were removed and ali the plots in this treatment were replanted on August 1 with plants from the original seedbed. These, without any further treatment, were placed as far as possible in the original dibble holes, and by mid August were successfully established, although their growth was very slow.

All the plants in the trial were harvested on October 2, and the information obtained is given in Tables 1 and 2.

TABLE 2

Analysis of Club Root Attack on the Cabbage Plants
(Number of Plants in = 136)

Treatment	No Attack	Attack on Lateral Roots		Attack on Tap Roots		Attack Indeterminate	Missing Plants
		Slight	*Severe*	*Slight*	*Severe*		
PCNB .. .. 30 lb per acre	25	6	2	5	78	12	8
PCNB .. .. 60 lb per acre	21	13	1	3	72	15	11
TCNB .. .. 8 lb per acre	19	0	0	1	97	8	11
Mercuric chloride 1 in 2,000	53	44	11	15	9	0	4
Control	15	4	1	1	107	3	5
Replants after PCNB. 1 in 320	118	2	0	1	2	13	0

Colhoun (1953) stated that in acid soils a maximum water-holding capacity of 70 per cent and a mean air temperature of 18–23°C provided favourable conditions for the development of club root. During this trial the soil was at pH6, the mean air temperature 7°–21°C, and although the maximum water-holding capacity was not calculated the heavy rainfall made the site extremely wet. Disease incidence was high in all treatments except where mercuric chloride was used. The number of healthy plants, marketable heads and weight of green matter obtained by this treatment was significantly higher at $p = 0{\cdot}01$ than that obtained by any other treatment.

It is interesting to note that in the U.S.A. Snyder, Leach, Sciaroni, Akesson and Parks (1955) successfully controlled club root with 1 in 2,000 mercuric chloride, and used a mechanical device for the accurate application of mercuric chloride to the dibble hole at transplanting. In the present trials, examination of the roots of the replants where PCNB liquid was used showed appreciable decortication not attributable to pest or disease, and suggests that the period of thirty-five days which elapsed between the treatment and planting out was insufficient, even in this very wet season, to remove all phytotoxicity. The replants were, however, reasonably free of infection, as is shown in Table 2.

Gallegly and Bishop (1955) obtained a good control of club root in the U.S.A. using 80 lb of PCNB per acre, but at 20 and 40 lb PCNB per acre the disease control was inadequate. In this pilot trial, the yield from plots treated with PCNB dust at a rate of 30 and 60 lb per acre, respectively, and from those treated with TCNB at 8 lb per acre, was not significantly different from the yield of the untreated plots.

I wish to thank Mr. W. E. Perry for his assistance.

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EFFECT OF POTASH ON POWDERY MILDEW IN WHEAT

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THERE is little documentary evidence to support the common idea that potash deficiency increases the severity of powdery mildew, *Erysiphe graminis*, on wheat. On Broadbalk field, where wheat has been grown every year since 1843, eye estimations of the incidence of this disease showed that it varied in different years from slight to severe, and was generally more severe on plots which received heavy nitrogen. The disease, slight to moderate in the previous three years, was exceptionally severe in 1934, when in the most severely infected plot (plot 8 in the first crop after fallow) most leaves were almost covered by the fungus; and the disease was consistently more severe in the first than in the second, third and fourth wheat crops after fallow.

In 1958, powdery mildew was very severe by the end of June on some plots deficient in minerals, relatively slight in others. Surveys were, therefore, made early in July on two adjacent sections which carried the first and third crops after a one-year fallow, on six plots which had all received the same amount of nitrogen with different combinations of mineral fertilizers, and on the dunged plot.

Disease incidence was measured by a method suggested by my colleague F. T. Last which was much more reliable than the previous eye estimations. The top leaf (leaf 1) and the next leaf down (leaf 2) were classified according to the proportion of their areas covered by mildew pustules. Fifty top and fifty second leaves on each plot were classified; the sample leaves were all taken from different plants, randomly scattered over the plots, excluding outer rows.

The Table shows that the older second leaves were the more severely infected. The disease was consistently most severe on three plots which received nitrogen and phosphate (plots 11, 12 and 14); the addition of sodium or magnesium (plots 12 and 14) had no apparent effect; it was moderately severe where nitrogen had been applied without phosphate (plot 10); relatively slight on the two plots which received potash as well as nitrogen and phosphate (plots 13 and 7); again sodium and magnesium (plot 7) showed no effect; and the disease was least severe on the two sections of the dunged plot. A statistical analysis of the counts of leaves with over 50 per cent of their area covered by mildew indicated that these differences were highly significant.

Powdery Mildew in Winter Wheat (Squarehead's Master 13/4) on Broadbalk Field, July 2–9, 1958

Plot No.	Treatment	Mean Percentage of Leaf Area Covered with Mildew			
		One Year after Fallow		Three Years after Fallow	
		Leaf 1	*Leaf 2*	*Leaf 1*	*Leaf 2*
Plots without Potash					
10	N_2	8·2	40·3	10·9	26·7
11	N_2, P	47·8	80·1	16·8	44·0
12	N_2, P, 3Na ..	41·4	74·2	14·6	35·5
14	N_2, P, 2½Mg ..	59·9	75·8	11·6	37·9
Plots with Potash					
7	N_2, P, K, Na, Mg	11·4	26·1	3·8	11·6
13	N_2, P, K	10·4	26·7	3·0	4·8
2	Dung	4·6	15·1	0·1	0·2

N_2 4 cwt sulphate of ammonia
P 3½ cwt superphosphate
K 2 cwt sulphate of potash
Na 1 cwt sulphate of soda
Mg 1 cwt sulphate of magnesia
} per acre

Dung 14 tons per acre (equivalent to about $2N_2$, P, 2½K).

Powdery mildew was thus most severe on plots which had unbalanced manurial treatments, receiving nitrogen and phosphate but no potash; the extra nitrogen made available by a preceding year under fallow increased the disease and enhanced the effect of potash in reducing it. The fourteen tons of dung applied annually (plot 2) provided an average of about twice as much nitrogen, about the same amount of phosphate and more than 2½ times as much potash as the mineral fertilizers. The lower incidence of powdery mildew can thus reasonably be attributed at least in part to the extra potash.

PEA LEAF ROLL, A NEW VIRUS DISEASE OF LEGUMES IN ENGLAND

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PEA leaf roll virus (Hopkins, 1957) has been reported from Germany (Quantz and Völk, 1954), Netherlands (Hubbeling, 1954, 1955) and Belgium (Roland, 1955). It has probably occurred for longer than these recent references indicate; a disease described from Germany (Böning, 1927) on *Vicia Faba*, L. closely resembles the condition caused by pea leaf roll virus in this plant.

Diseased broad bean plants showing the characteristic chlorosis and rolling of the tip leaves were first noticed at Rothamsted in 1955, and similarly affected plants were seen again in 1956 and 1957. The disease has also been seen in these years in many crops of peas and field beans in south-eastern England. The first transmissions of the disease were obtained in 1956, when the aphid *Macrosiphum pisi* (Kltb.) was fed for several days on field beans, and subsequently transferred to peas and broad beans which had been raised under glass. Earlier attempts to transmit the disease by sap inoculation, and by *M. pisi* which had fed for only a short time on infected plants, were unsuccessful.

HOST RANGE, SYMPTOMS AND TRANSMISSION

Quantz and Völk (1954) reported that *V. sativa*, L., *V. narbonensis*, L., *Medicago sativa*, L. and *Pisum melanocarpum* were hosts of the pea leaf roll virus. De Fluiter and Hubbeling (1955) found that aphids collected from lucerne, white clover and, occasionally, red clover transmitted the virus to peas on which they fed for long periods. At Rothamsted, successful transmissions were made from naturally infected field beans to healthy peas and broad beans, but no other sources were tested. However, on the Rothamsted farm in 1956, in crops of field beans sited near lucerne, it was clear that the incidence of pea leaf roll decreased with increasing distance from the lucerne, suggesting that the lucerne was infected and acted as a source of infection for the bean crop. Similar gradients of infection have also been observed in the Netherlands (Hubbeling, 1955).

The symptoms produced by the pea leaf roll virus in field and broad beans, *V. Faba*, L., growing in the open, are definite and characteristic. The first signs show by the topmost leaves beginning to roll and turning slightly yellow; in later stages of the disease the upper half of the plant usually has bright yellow, rolled leaves, which are thickened and brittle (Plate 1). The plants are stunted, considerable defoliation occurs and many growing points become necrotic. In peas the symptoms are similar but less severe; diagnosis is sometimes difficult because infections with *Fusarium* spp. also make pea plants chlorotic, but such plants usually wilt. Under glasshouse conditions, the symptoms appear in about a month, and in broad beans are very distinct, usually taking the form of a green vein banding. Peas show only a transient chlorosis but infected plants are always severely stunted.

Pea leaf roll virus was not transmitted by the usual methods of sap-inoculation, and was transmitted only with difficulty by aphids. *Myzus persicae* (Sulz.) and *Macrosiphum pisi*, the only aphids tested as vectors, both transmitted

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when allowed to feed on infected plants for three days and then on the test plants for two days; and *M. pisi* transmitted the more often. Using ten aphids per test plant in these feeding conditions, about 30 per cent of the test plants became infected in the glasshouse, and up to 90 per cent in field cages.

EFFECTS ON YIELD

Preliminary attempts to estimate effects on yield were made in three crops of field beans at Rothamsted in 1956. Twenty-five infected plants were selected at random early in the season in each of the crops; their yields were measured at harvest and compared with those of the nearest healthy plant in an adjacent row.

Effect of Pea Leaf Roll Virus on Yield of Field Beans

	Mean Weight of Beans per Plant		Mean Number of Pods per Plant		Weight of 100 Beans	
	Healthy g	*Diseased* g	*Healthy*	*Diseased*	*Healthy* g	*Diseased* g
Field 1	13·7	6·8	10·0	6·0	41·7	37·9
Field 2	22·4	10·7	14·0	8·0	47·4	42·5
Field 3	24·5	9·4	17·0	8·0	50·1	46·1
Mean	20·2	9·0	13·7	7·3	46·4	42·2

The infected plants died earlier than the healthy ones, and they showed considerable necrosis of the tops relatively early in the season. The Table shows that infection decreased yield by about 50 per cent, rather by reduction in the number of pods formed than of effect on bean size.

In 1956, the first infected bean plants were seen in a crop on the Rothamsted farm on July 4, and by the end of the month 5 per cent of the plants showed symptoms. It then became impossible to make accurate counts because the plants were very tall, and assessments of plant populations were further complicated by lodging. Counts were made, however, on a crop at Cambridge up to the middle of August, when 20 per cent infection was recorded. The incidence was probably higher than this at the end of the season, but the state of the plants in the field prevented estimates being made.

It is interesting to record that attacks of the chocolate spot fungus, *Botrytis fabae* Sardiña, were more severe on leaf-roll infected beans than on healthy ones. This was observed consistently both at Rothamsted and Cambridge.

The photographs were taken by V. Stansfield, F.R.P.S.

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PEA LEAF ROLL ON FIELD BEANS

Photos: Rothamsted Experimental Station

Left: Bean plant showing advanced symptoms. *Right:* Bean leaf showing typical rolling and chlorosis.

PLATE I

Colour-banding, or "rugby-stocking" effect in wheat seedlings from seed sown at a depth of 6 inches. Nord Desprez, sown at Cambridge on December 21, 1952; photographed February 19, 1953.

PLATE II

STEM AND BULB EELWORM ATTACKING SWEDES

1. Infested plant, showing thickening and malformation of leaves.

2. Infested plant, showing killing of growing point.

PLATE III

BROOMRAPE ON RED CLOVER

1. General view of broomrape growing on red clover.

Photos : Welsh Plant Breeding Station

2. Close-up of broomrape on red clover.

PLATE IV

EXPERIMENTS ON THE CONTROL OF BROOMRAPE IN RED CLOVER

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SEVERAL fields at the Frongoch farm of the Station are heavily infested with broomrape, *Orobanche minor*, which has caused considerable damage and casualties among red clover spaced plants. White clover is attacked to a lesser extent and it has been observed that certain clones of lucerne are also susceptible. It is thought that the primary infection took place in the 1920s with a seed lot of crimson clover, and efforts made to control the parasite, by weeding, have not been effective.

Broomrape (Plate IV) is a total parasite found growing on the roots of red clover and other hosts, and consists of little more than an erect flowering stem with a swollen underground base attached to the host roots. The flowers produce a large number of very small seeds which fall to the surface of the soil, and will normally germinate only when they are near to a host root; this has been shown to be influenced by a chemical stimulant released from the root (Chabrolin, 1934). Brown, Greenwood, Johnson, and Long (1951) have shown that the exudate from linseed roots is a potent source of the chemical stimulant, although linseed itself is not normally parasitized. These results suggested that growing linseed on the infected land might effectively reduce the parasite population. The presence of the linseed roots should encourage the seed of the parasite to germinate but in the absence of a suitable host the germinated seedlings would die. Blanchard (1952) has effectively controlled *Orobanche speciosa* infection in peas by dusting the seed and drills with "soprasan" mercurial seed dressing. Two experiments were therefore set up to see if it was possible to control broomrape on a field scale. A pilot experiment was carried out in boxes to study the effect of linseed, and this was followed by a field experiment on an area severely infested with broomrape.

PILOT EXPERIMENT

In the pilot experiment, set up on April 28, 1952, there were 144 boxes arranged in a design in which the number of sowings of linseed was confounded with blocks. The treatments were:

Depth of Broomrape Seed	*Linseed Rates*	*Number of Linseed Breaks*
On surface	Nil	One
2 cm below surface	40 lb per acre	Two
8 cm below surface	80 lb per acre	Three
At all 3 levels	160 lb per acre	

In the sowing of the *O. minor*, a small amount of the seed was scattered on the surface of the sterilized soil in the boxes, and calculated quantities of soil were added to cover them to the desired depths.

Germination of the linseed was good, and warm conditions helped the seedlings to develop strongly. Flowers began to appear about the middle of July when the 3-break linseed treatment was cut and resown. The linseed was cut near the surface, and the top soil slightly roughened with a stick before resowing. The 2-break linseed treatment was likewise cut down and resown on August 6. All the linseed was cut at the end of October.

All boxes were sown with the test crop, S. 123, red clover, on April 2, 1953 and this was cut on August 6, when one broomrape was noted. The boxes were kept under observation and the heads of broomrape removed as they appeared.

A few spikes continued to appear up to January 1954, but with one exception all were in boxes that had not had the linseed pre-treatment:

	1953				1954
	Sept. 17	*Oct. 10*	*Oct. 20*	*Dec. 14*	*Jan. 1*
Number of broomrape spikes removed at each date	5	7	5	12	11

The last spikes appeared to have been killed by a hard frost which occurred on January 5. Broomrape has, therefore, appeared as late as December; and some of these were first appearances in particular boxes, so that they could not have developed from the bases of broomrape previously removed.

Broomrape was rather late in appearing in 1954 and none was recorded before August 3; the clover was cut on August 12. Counts of broomrape were made on four dates: August 14, September 11, October 4, and October 21. The largest number appeared in the boxes that had not received the linseed pre-treatment, but six out of these thirty-six boxes gave nil counts. Of the remaining 108 boxes, all having received the linseed pre-treatment, 77 gave nil counts. The main difference appeared in a comparison of results from boxes with and without the linseed pre-treatment as is shown in Table 1.

TABLE 1

Pilot Experiment. Effect of Pre-cropping with Linseed.

	Linseed Seed Rate (*lb per acre*)				
	0	*40*	*80*	*160*	*Total*
Broomrape counts					
1953	39	—	1	—	40
1954	104	22	19	42	187
Total	143	22	20	42	227
Red clover, green weight (g) ..	1,845	3,458	3,680	3,776	—

Growing linseed caused a marked decrease in broomrape counts, but the actual seed rate of linseed had comparatively little effect. The weight of clover in the plots previously sown with linseed at different rates was very similar, and almost twice that of the control. The poor weight of the clover in the control plots could be attributed to the deleterious effect of the parasite, as it caused a considerable number of casualties among the plants that were attacked during the autumn of the seeding year. There was little difference in the broomrape counts for sowing depths of 0, 2 and 8 cm, but where it was sown at each of the three levels, by mixing it with the top 8 cm of soil, the count was lower:

	On Surface	*2 cm deep*	*8 cm deep*	*Mixed with top 8 cm*
Broomrape counts	77	65	62	25

Increasing the number of linseed breaks did not make much difference to the counts; which were 33 with one break, 17 with two breaks and 31 with three breaks. The results of the pilot experiment indicated that pre-treatment with linseed was effective in reducing the incidence of broomrape, but increasing the number of linseed breaks caused little further improvement.

FIELD EXPERIMENT

This experiment was set up on a field known to be severely infested with broomrape. The experimental area was sown with S. 123, red clover, in the

summer of 1953, to get information on the uniformity of broomrape distribution. The red clover crop was cut in mid June 1954 when the first broomrape spikes were noticed; the spikes appearing in the aftermath could then be counted with ease. Plots, 15 ft × 15 ft, were marked out and all the spikes were pulled up and counted on July 13, 1954. The counts ranged from 3 to 784 per plot (Plate IV). Broomrape continued to appear, and a further count on three traverses only on August 6 gave numbers ranging from 29 to 824. No attempt was made to count individual plants so that the spike counts would represent fewer plants. At the second count some of the spikes may have grown from the bases of previously picked plants, but in other instances the whole swollen base of the broomrape would come away in one piece. After this count the plots were mown over to prevent reseeding and the area was later ploughed.

The pre-treatments with linseed were commenced in 1955; the results obtained in the uniformity trial were given consideration in arranging the plots. Twelve main plots were delimited each consisting of six of the plots used in the uniformity counts. Three treatments were adopted: fallow; one break and two breaks of linseed. The linseed was sown in April and gave a good take. In the two-break treatment the first crop was mown and removed at the beginning of July; the land was ploughed and resown immediately. There was a considerable delay in the germination of this sowing because of the drought that lasted until mid August. The linseed on well consolidated soil in the tractor tracks grew immediately, and had reached a height of about 12 inches before the main bulk commenced growing. This crop remained green well into November when it was damaged by frost. The single break linseed was harvested for seed when it was ripe; the whole area was then ploughed and cultivated ready for sowing the test crop of S. 123, red clover.

In July 1956, each of the main plots was split into six sub-plots, which coincided with the original plots in the uniformity trial. Red clover, S. 123 was sown at the rate of 10 lb per acre throughout; the treatments of the sub-plots being: (1) seed dressed with excess soprasan (surplus screened off) and broadcast; (2) seed dressed with soprasan and sown in drills 6 in. apart, with soprasan also dusted in the drills; (3) seed dressed with excess agrosan G. (surplus screened off) and broadcast; (4) seed dressed with agrosan G. and sown in drills 6 in. apart with agrosan G. also dusted in the drills; and (5) and (6) controls, with untreated seed, broadcast.

TABLE 2

Field Experiment. Mean Counts of Broomrape per Plot in 1954 Before Treatments, and in 1957 After Treatments

Seed Treatment	One Linseed Break		Two Linseed Breaks		No Linseed	
Red Clover, S 123	*1954*	*1957*	*1954*	*1957*	*1954*	*1957*
Dressed with soprasan and broadcast	198	144	150	128	227	258
Dressed with soprasan and sown in drills	112	157	279	115	209	134
Dressed with agrosan and broadcast	131	154	273	79	188	163
Dressed with agrosan and sown in drills	188	258	101	72	292	237
Controls: seed untreated	264	176	230	140	191	195
and broadcast	316	254	148	75	228	202
Means: all treatments and controls	202	191	197	102	223	198

The results are given in Table 2. An analysis of variance showed no significant differences in the counts of broomrape as a result of either linseed pre-treatment or seed treatment. This was in spite of the fact that after two linseed breaks the mean broomrape counts were reduced by 48 per cent, in comparison with a reduction of 11 per cent where no linseed was sown. The lack of statistical significance is the result of large variation in the broomrape count from block to block, and the few degrees of freedom for the error variance in the main plot analysis. Block differences were significant, however, and the percentage changes in the counts in the four blocks, as a result of the linseed treatment were:

Block I	Block II	Block III	Block IV	Mean I–IV
−51·5	−72·1	+38·5	−30·0	−21·0

The larger decrease in block II than in blocks I and IV was presumably due to the effect of drought in the spring of 1957 when the growth of clover was very poor in block II. In block III, however, the counts increased considerably, and no immediate explanation of this is available.

The results indicate that no spectacular decrease in the numbers of broomrape can be obtained by linseed pre-treatment. This is perhaps not surprising as the very large numbers of seed produced by the parasite are probably distributed in infected soil to a depth of at least eight inches by cultivations, and may be washed down to a greater depth by drainage water. Ploughing brings these dormant seeds to the surface where they can remain until conditions are favourable for germination. It is not known whether all seeds at all depths are affected by the root stimulant, but it would seem more likely that at least a proportion of the seeds would remain dormant, and that seeds at lower depths might not be affected at all. If this were so, pre-treatment would have to continue for several breaks and it might be possible to develop a rotation with other crops which also produced the stimulating factor.

The lack of control obtained from the mercury seed dressings of red clover, compared with Blanchard's results on peas, is probably due to the longer period that the red clover plant occupies the ground. Peas occupy the ground for only a short period and a temporary inhibition of infection from the broomrape might be enough to prevent the parasite from getting established.

SUMMARY

In a pilot experiment carried out over the period 1952–54, with sowings of broomrape seed in boxes, cropping with linseed reduced the number of broomrape plants and nearly doubled the green weight of red clover produced in a subsequent sowing. In a replicated field trial set up in 1953, on a field known to be heavily infested with broomrape, there was some reduction of the broomrape counts in S. 123, red clover, as a result of pre-cropping with linseed, but even with two linseed breaks the reduction was not significant at the 5 per cent probability level. Treatment of the clover seed with mercurial seed-dressings had little or no effect on the control of broomrape.

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METHODS FOR THE DETECTION OF VASCULAR WILT PATHOGENS IN CARNATION CUTTINGS

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THERE are four vascular diseases of the perpetual flowering carnation (*Dianthus Caryophyllus*): Verticillium wilt (*V. cinerescens* Wollenw.), Fusarium wilt (*F. oxysporum f. dianthi* (Prill and Delacr.) Snyder and Hansen), bacterial wilt (*Pseudomonas caryophylli* (Burkh.) Starr and Burkh.) and slow wilt (a strain of of *Erwinia chrysanthemi* Burkh. *et al.*). Verticillium wilt is the most serious disease in England although Fusarium wilt and slow wilt have caused serious losses. Bacterial wilt has been recorded only once (Lelliott, 1956). In the U.S.A., Fusarium wilt and bacterial wilt are serious diseases, but Verticillium wilt and slow wilt have not been recorded there. All four diseases may be carried in cuttings taken from infected plants and, since the diseases can exist in a latent form, it has been extremely difficult to select healthy plants from which cuttings can be taken.

Dimock (1943) devised a method for detecting Verticillium wilt (*V. albo-atrum*) in chrysanthemum cuttings which proved so successful that the disease ceased to be one of great importance. This method has been adapted with various modifications for use in carnations by Dimock (1948), Forsberg (1950), Guba (1952) and Tammen, Baker and Holley (1956) in the U.S.A., by Hellmers (1955) in Denmark and by Glasscock (1956) in England. Essentially the method consists of surface sterilizing the lower part of the stem of the cutting and then taking several transverse sections and transferring them to a culture medium. After an appropriate time the culture tubes are examined and if any microbial growth is present then the corresponding cutting is rejected. The method is, therefore, one for screening out cuttings infected with the pathogens and not for the identification of specific pathogens.

During a visit to Cornell University in 1955–56 a study was made of methods for testing carnations. The method is primarily intended for use by a grower on his nursery, and this work, the results of which are given below, was carried out with this in mind. Particular attention was paid to surface sterilants, culture media and the time and temperature of incubation.

INOCULATION OF CUTTINGS FOR TESTS

It is not possible to obtain a sufficient number of cuttings, known to be infected, from infected plants; so cuttings were inoculated by placing freshly cut bases in suspensions of fungal spores or bacterial cells of the respective pathogens. In the cases of *F. oxysporum f. dianthi* and *Ps. caryophylli* extensive tests were made to find optimum conditions of inoculation which would provide cuttings with the minimum concentration of inoculum, applied for the shortest time, to give uniform results. It was thought that cuttings inoculated in such a way might emphasize any differences in treatments which might not show when higher concentrations of inoculum, or longer times for inoculation, were used. For *V. cinerescens* and *E. chrysanthemi* less extensive tests were made. The placing of cuttings for one hour in a suspension containing, respectively, 5,000 spores per c.c. of *F. oxysporum f. dianthi*, 10,000 spores per c.c. of *V. cinerescens*, 10,000 cells per c.c. of *Ps. caryophylli* and a standardized but unknown number of cells of *Erwinia chrysanthemi* was found to be the best procedure for inoculation, and it was used in subsequent tests. The spore concentration was

determined by a haemocytometer and bacterial cell concentration by determining the turbidity of suspensions in a photometer. For *Ps. caryophylli* the turbidity had been previously related to the number of bacterial cells by making a dilution series and counting colonies on agar plates. Under these conditions the pathogens were drawn into the basal 3–4 cm of the cutting. It is interesting to note that when times longer than one hour were used the bacterial pathogens could be found at much higher levels in the cuttings, whereas the fungal pathogens were not found at much higher levels even after leaving the cuttings 48 hours in the suspensions. Later more limited tests with naturally infected cuttings showed complete agreement with the tests carried out with the artificially inoculated cuttings. In carrying out these tests transverse sections about 1 mm thick were made through the stems of inoculated cuttings and the sections transferred to the appropriate medium in culture tubes.

SURFACE STERILIZING MATERIALS

Several materials were examined including mercuric chloride (1 in 1,000) followed by washing in sterile water, clorox (a proprietary material containing 5·25 per cent sodium hypochlorite) used as 20 per cent clorox, lysol (1 in 100), and dipping in alcohol and flaming. Mercuric chloride and clorox were found to be the most efficient, but clorox was preferred because it is easier to use. Neither of these chemicals had any marked effect on the pathogens in the vascular tissues when stems, either as the basal part of a cutting or as an isolated internode, were immersed in the solutions for five minutes. Even when the stems were immersed in the solutions for thirty minutes, no serious effects were observed except when the base of cuttings inoculated with *Ps. caryophylli* were placed in mercuric chloride. In this case, the chemical sometimes killed bacteria up to 1·5–2·0 cm from the base of the stem. However, it is unlikely that cuttings would be placed in solutions for such a long period. When periods of five minutes are used, the removal and discarding of the first 1 cm of stem, before plating out sections, would ensure that the surface sterilizing agent would have no effect on the organisms in the vascular tissue. Further tests showed that under most conditions, 20 per cent clorox for one minute and 5 per cent clorox for five minutes gave satisfactory results.

Occasionally a bacterium which was not identified, but was shown to be a spore-former, was not eradicated, when present on stems, by immersion in 20 per cent clorox or 0·1 per cent mercuric chloride for ten minutes. This contaminant (and possibly other spore-formers) did not occur sufficiently frequently for a search for a better bactericide (and as good a fungicide) to be made. Occasionally such a contaminant may prove a nuisance in a culturing method, but could probably be tolerated.

The use of a wetting agent with clorox and mercuric chloride gave consistently better results. Several wetting agents, including household detergents, were tried and gave good results, but triton X 100 used as one drop in 100 ml consistently gave the best results.

MEDIA

Those used were difco potato dextrose agar (P.D.A.), freshly made potato dextrose agar, difco nutrient broth + 1·5 per cent dextrose and potato dextrose broth. The tests were carried out with the four pathogens at their respective optimum temperatures for growth. The four pathogens grew well in all four media. Usually the pathogens were detected in the broth media a day before they were detected on the agar media, but this is not significant for the purpose of the culturing technique. On some occasions when the agar media were not freshly prepared, the sections tended to dry out, especially those near the top

of the tube, and bacterial growth was often poor. Because of these slight differences, but particularly because the transfer of sections to the media is so much easier, the use of broth is preferred. Both broth media gave excellent results. The identification of organisms in broth is often difficult or impossible, but this is not important, since the culturing technique is, in fact, a screening method. If the identification of the organism is important, then the use of agar media would be more convenient. Better results were obtained with freshly prepared P.D.A. than with difco P.D.A., and *Ps. caryophylli* was always identified more readily on the freshly prepared P.D.A.

TIME AND TEMPERATURE OF INCUBATION

Sections taken from cuttings inoculated with the respective pathogens were placed on P.D.A. slopes and then incubated at temperatures of 15°, 18°, 22·5°, 28°, 31° and 35°C. In some cases 15° and 35°C were not used. The time (in days) taken for growth of the pathogens to be seen clearly, and without undue examination, around most of the sections at the various temperatures was as follows. The time at which growth was first seen is not given.

	15°	*18°*	*22·5°*	*28°*	*31°*	*35°*
F. oxysporum		3–5	2	2	2	14
V. cinerescens	7–10	7–8	4–5	5–7	no growth	
Ps. caryophylli		10–14	5	2	2	2
E. chrysanthemi	6	5	3	2	2	

A temperature of 22·5–28°C would be suitable for all four pathogens. Three of the pathogens could be seen clearly after three days, but *V. cinerescens* usually required 5–7 days. It was also noted that occasionally the growth from some sections was detected some 3–4 days after that around other sections. It was, therefore, decided that cultures must be left at least seven days and preferably, with a margin for safety, ten days at a temperature of 24–25°C before examination. Later experiments with nutrient broth + dextrose gave very similar results.

DISCUSSION

This method of testing carnation cuttings for vascular pathogens depends on the pathogens being continuous in the vascular tissue so that, if no microbial growth occurs in the culture tube from the sections, one can assume that the tissue above is free from the pathogens. The small amount of information available (e.g., Wickens, 1935) suggests that the pathogens may not always be continuous. However, as in the case of chrysanthemums, the success of the method with carnations in Colorado (Baker, 1957), and also in my experience, suggests that the distribution of the pathogens is such that at least a very large majority of infected cuttings are screened out. In the last two years, about 6,000 cuttings have been tested on three nurseries in the Lea Valley, and none of these plants have been affected by a wilt disease. The application of the method in successive years to the same stock would, in any case, soon ensure that all the plants were free from the vascular diseases.

In methods previously described sections are taken from one internode. By taking sections from two internodes the chances of detecting the pathogens would be increased. It is also suggested that a section should be taken through the intervening node as Mr. R. A. Lelliott (*in lit.*) and I have observed, that there is a tendency for bacteria, in the case of slow wilt, to be concentrated at the nodes.

The Table above shows the importance of the correct time and temperature of incubation. Hellmer's (1955) suggestion of incubation at 28°C for 3–4 days would almost certainly fail to screen out cuttings affected by Verticillium wilt. Nutrient broth + 1·5 per cent dextrose and clorox were found to be the best

and most convenient culture medium and surface sterilant, respectively. Because of the occasional occurrence of a high proportion of cultures with contaminants, especially bacterial, the use of a more selective medium might be considered, but there is a serious objection to this. The testing method should be one likely to detect any new vascular pathogens which might occur, and not only those known already. In this connection it is interesting to note that slow wilt appeared after the testing method had been first suggested. It is probably far better to tolerate the occasional high losses because of contaminants, and to attempt to reduce these by taking cuttings from the upper part of clean, carefully grown, stock plants where dead leaves, etc. are absent. It is important to test the cuttings immediately since it has been found that keeping them in polythene bags, where humidity is high and condensation frequently occurs, often results in a very high incidence of bacterial contaminants.

From this work, and the practical experience gained in the various culturing methods, a method has been devised which can be used fairly easily by a grower and which will detect all four pathogens. A detailed account of this has been given by Jenkins (1958). In brief, the selected cuttings are two internodes longer than normal. About 5–10 of these are placed in 20 per cent clorox (plus wetting agent) so that the two lower internodes are covered for five minutes. (In England a proprietary hypochlorite solution "chloros" used as 20 per cent chloros has given satisfactory results.) The cuttings are then placed between two large sheets of absorbent paper. Each cutting is removed in turn and placed on a smaller piece of absorbent paper. Using a sterile razor blade or scalpel, transverse sections are taken, two from each internode, and one through the intervening node, and transferred to a tube containing difco nutrient broth + 1·5 per cent dextrose. The tube is labelled and placed in an incubator at 24°C. The cutting is placed in a polythene bag just big enough to contain one cutting, labelled, and stored in a refrigerator at a temperature in the range of 0–4·5°C. After ten days the tubes are examined and where microbial growth is noted, the corresponding cutting is rejected. The remaining cuttings are assumed to be free from vascular pathogens and are grown under very carefully controlled conditions as the nuclear stock plants.

In practice it has been found best to test a relatively small number of cuttings so that proper care can be taken. These nuclear stock plants are then multiplied in two or three stages to provide cuttings for the flowering beds.

SUMMARY

An examination of methods for the detection of Verticillium wilt, Fusarium wilt, bacterial wilt and slow wilt in carnation cuttings has shown that the most convenient and best nutrient medium and surface sterilizing agent are nutrient broth + dextrose, and clorox (sodium hypochlorite). The tubes should be incubated at 24°C for ten days. A method which has been successfully used by growers is outlined.

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FIELD SAMPLING AND LABORATORY EXAMINATION OF CEREAL ROOT EELWORM CYSTS

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ANSCOMBE (1950) has reported on the co-operative examination of factors involved in the field sampling of potato root eelworm, *Heterodera rostochiensis* Wollenweber, while Fenwick (1940) has described what is now the most commonly used technique for the extraction of cysts of this species from soil.

Observations on cereal root eelworm, *Heterodera major* O. Schmidt, (Hesling, 1956) have suggested that its cysts might behave rather differently from those of potato root eelworm; for example, in the laboratory, newly formed cysts tend to sink in water and some cysts may be found at the bottom of the Fenwick can. In discussions of a sub-committee of the N.A.A.S. Conference of Advisory Entomologists in 1955 it was decided to examine the effectiveness of methods being used in field sampling and laboratory examination of *H. major*, and a study group was set up for this purpose, consisting of E. B. Brown, R. Gair, J. J. Hesling, S. W. Rolfe and Miss C. M. G. Duthoit, together with the writers of this paper. The results of the work are summarized below.

LABORATORY EXPERIMENT: TECHNIQUES

Ten soil samples, of widely differing types, but all known to be infested with *H. major*, were collected in late spring from various parts of the country and, after being sub-divided, each was examined at the N.A.A.S. centres at Cambridge, Cardiff, Derby and Wolverhampton, as well as at Rothamsted Experimental Station and the Plant Pathology Laboratory, Harpenden. As the samples were

all taken in the spring, the cysts recovered had been formed the previous season, or earlier. Each centre was asked to use its normal method of examination to recover cysts of *H. major* from these soils. At all centres this involved washing 200 g of an air-dry soil sample through a coarse sieve which allowed cysts to pass through into the Fenwick can. Two such 200 g sub-samples of the soil from each of the ten sites were examined at each centre. The float was collected into a finer sieve, which retained the floating cysts, from which it was either washed directly on to a filter paper, or re-floated in a 1-litre flask before filtering. Cysts were then collected by various means and counted. Details of the techniques used at the different centres are summarized below.

In most cases, the bulked soil sample from the field had been sieved before distribution to the six centres co-operating in the experiment, and the soil was re-sieved at each centre. For this, a coarse sieve ($\frac{1}{8}$–$\frac{1}{4}$ in. mesh) was used at all centres except Derby, where a 20-mesh sieve was used. Methods of crushing the soil varied, Derby using a wooden mallet and Cardiff a metal roller. The soil was air dried in aluminium or tinplate trays or, as at Rothamsted, in cotton bags. At Derby it was dried slowly over electric heating elements. Sub-samples were extracted from the bulk by "coning and quartering"; at Cardiff, this was done by hand on a sheet of paper and a single 200 g sample was weighed out of about 500 g remaining at the last portion. The whole sample was then re-mixed as before and a second 200 g sample was taken. At Wolverhampton the soil was coned and quartered about twenty times to produce two sub-samples; at Rothamsted the mixing was done with a trowel, both 200 g samples being taken from the same mixture, and at Harpenden the soil was mixed in the drying tray.

The metal used in the construction of the Fenwick apparatus varied from centre to centre but this did not appear to affect the results obtained. The size of the top sieve and that used for collecting also differed. Thus for the top sieve Rothamsted used 16 mesh; Harpenden, Derby, Cambridge and Cardiff 20 mesh and Wolverhampton 30 mesh. With a wire of s.w.g. 28 and 20 meshes per inch, the opening width is 0·894 mm which is rather smaller than the maximum recorded *H. major* cyst. A 30 mesh sieve is not therefore to be recommended. For the collecting sieve, 100 mesh was used at Cardiff, Rothamsted and Harpenden for some soils (80 mesh for others); 80 mesh was used at Cambridge and 60 mesh at Derby and Wolverhampton. (The normal 100 mesh B.S. 410/1943 has a wire of s.w.g. 42 and an opening width of 0·152 mm.)

At Cardiff, Rothamsted and Wolverhampton, the residues from the collecting sieves were shaken up with water in a 1-litre flask and allowed to stand for 2 to 5 minutes before decanting on to filter paper or bolting cloth. At Rothamsted the float was dried before being poured into a flask half full of water. The floats were then examined under a microscope and cysts collected. Cardiff and Rothamsted used a Fenwick counting tray (Goodey, 1957) and aspirator (Hesling, 1952), and Wolverhampton collected cysts from filter paper. At Cambridge and Harpenden, cysts were collected directly from filter paper without refloating in a flask, while at Derby the residues were refloated in a developing dish and cysts collected from the surface with a camel-hair brush. This last is hardly to be recommended and may possibly account for the low average figure of cysts recovered at this centre.

In addition to the routine examinations described above, the residues from the Fenwick cans were washed into the collecting sieves of the apparatus and transferred to filter paper or bolting silk, or (at Wolverhampton) to a drying tray. The contents were then air dried and divided into portions of 20–50 g which were shaken up with saturated magnesium sulphate solution. The floats were decanted into filter papers and the cysts recovered and counted.

RESULTS OF THE LABORATORY EXPERIMENT

Averages for total cyst counts per 200 g of soil for each site were:

Group 1: Less than 100	Glamorgan, 42; Nottingham, 66; Shropshire, 51; Staffordshire, 51.
Group 2: 100–299 ..	Cambridgeshire, 200; Hampshire (Longwood), 200.
Group 3: 300–499 ..	Denbigh, 327; Lincolnshire, 436; Essex, 450.
Group 4: More than 499	Hampshire (Whitchurch), 728.

Bulked on a N.A.A.S. Regional Centre basis the average number of total cysts per 200 g of soil was: Cardiff, 278; Derby, 206; Cambridge, 303; Wolverhampton, 264. Similar experiments at Rothamsted and Harpenden gave 224 and 258 cysts per 200 g respectively.

The analysis of the site count data after square-root transformation was:

Factor	df	MS
Between sites	9	615·4
Between centres	5	21·8
Centres × sites	45	4·4
Between sub-samples within sites	60	0·72

There are significant differences between the population estimates for the same sites made at different centres, the determinations at Rothamsted and Derby being rather lower than those at the other centres. Despite such differences, however, if the sites are divided into four groups according to population as indicated above, the sites would have been allocated similarly to these groups on the basis of the determinations at all of the centres. For practical purposes discrimination within such groups would often be unnecessary, except possibly when the cyst population is low.

The estimated variance between the square roots of total counts on duplicate 200 g sub-samples differed from centre to centre, but there is insufficient data to determine more than an average value (0·72), which is almost three times the Poisson variance (0·25) which would be obtained if mixing was perfect and there were no errors of counting.

Any improvement in the mixing of bulk soil samples, and in subsequent handling, by which the sub-sampling errors could be made to approach the theoretical value, would be a considerable practical advantage and would enable estimates of equal accuracy to be obtained from sub-samples a half to one-third of the present size. Counts made on a larger set of duplicate sub-samples at one centre indicate that, in certain circumstances, the theoretical minimum sub-sampling error may be approached by the use of a thorough coning and quartering technique.

There were large differences between the average percentages of cysts left in the sediment at the bottom of the Fenwick can for different sites. In addition, there were significant differences between centres in the average losses from this source, and the differences between centres varied from site to site. At most sites the average loss was about 5 per cent of total cysts. At Corntown, Glamorgan and Rodbaston, Staffs., losses were greater, averaging 11 per cent and 19 per cent respectively. At Wroot, Lincs., the average loss was only 2 per cent. Averaging over all sites, losses at most centres were about 6 per cent, those at Harpenden being consistently greater.

In Table 1 the average per cent losses at the different sites may be examined in conjunction with the total cyst counts and various characteristics of the soil samples. Clearly the percentage losses tend to be higher at sites with small total counts. Percentage losses are also greater when the percentage of "viable" cysts (that is, cysts with contents) is low—it has been observed that a greater proportion of non-viable cysts are lost; only 20 per cent of all cysts recovered

TABLE 1

Percentage Cyst Loss, Cyst Counts and Soil Characteristics

Site and Soil Type	Fine Sand *per cent*	Coarse Sand *per cent*	Mean Cysts *per 200g*	Mean Cysts Lost *per cent*	Viable Cysts *per cent**
Corntown, Glam Light loam over carboniferous limestone	52·8	9·5	42	10·7	47
Ruthin, Denbigh Light loam over clay-with-flint series ..	36·6	20·2	327	5·6	52
Gleadthorpe, Notts Bunter pebble beds	9·8	78·3	66	6·3	60
Wroot, Lincs Peat and sand	8·8	24·6	436	1·8	55
Hodnet, Shropshire Bunter sand	11·5	52·5	51	7·0	45
Rodbaston, Staffs Clay/sand	32·3	48·0	51	19·2	13
Duxford, Cambs Silty loam	48·3	22·7	200	7·3	52
Audley End, Essex Organic calcareous limestone	29·8	14·3	450	2·7	17
Whitchurch, Hants Limestone	47·3	6·7	728	4·5	30
Longwood, Hants Chalky loam	41·4	4·0	200	5·0	31

*Figures from Cardiff only.

from the sediment in the can were viable. Information from a much larger number of sites would be necessary to demonstrate convincingly any relationship between soil characteristics and cyst losses; but it is suggested that, for given population levels, percentage losses are least on soils with small amounts of clay or silt particles which adhere readily to the cysts.

FIELD HETEROGENEITY

Data from pairs of 50 g soil samples taken at a distance one yard apart from each of twenty (or occasionally ten) evenly spaced stations on randomly located traverses are available for ten fields; it is assumed that the stations were located effectively at random within the fields. Each 50 g sample was examined separately in the laboratory, using the techniques described above.

The cyst populations of the sampled fields varied from 0·1 to 1·0 cysts per gramme and, as might be expected, the estimated variances between cyst counts, based on duplicate 50 g samples, were greatest for fields with heavy populations. Although information from many more fields would be needed to establish the relationship between average variance and mean cyst count, the data are in reasonable agreement with the simple working assumption that the average variance is proportional to mean cyst count, thus:

$$\sigma^2 = am$$

where m is the cyst population per gramme, σ^2 is its variance, and a is a constant for the type of sample specified. If this relationship holds, the average variance of the square roots of the counts is independent of the mean for samples of a given size. A combined analysis of variance on the square roots of the sample counts from ten fields took the following form:

Factor	df	MS
Between stations	170	2·14
Within stations	180	1·22

This shows that samples one yard apart resembled each other more closely than samples widely distributed over the fields; indeed, there was evidence of heterogeneity in nearly all the sampled fields. Clearly, if one is interested in following changes in cyst population in a field, it will be advantageous to sample from the same points on each occasion. This may be expected to increase the accuracy of estimated changes in population by 30 per cent or more compared with independent random sampling on each occasion.

ACCURACY OF CYST POPULATION ESTIMATES

Several factors influence the accuracy with which the cyst population density of a field is determined, and they may be considered under two headings:

1. Obtaining a representative bulk sample of soil from the field.
2. Subsequent handling of the soil, laboratory examination, and cyst counting on a sub-sample.

In order that the cyst population of the bulk sample may represent that of the field as a whole, it is necessary both that a sufficient quantity of soil should be sampled, and that the soil should be sampled from a sufficiently large number of randomly located points within the field. Table 2 shows that, for a given total weight of soil, the standard error of the estimated population per gramme is smallest when the sample is made up of approximately equal weights of soil taken from a large number of randomly located points. It is not possible, however, merely by increasing the number of sampling points, to increase indefinitely the accuracy of a population estimate obtained by examining a small weight of soil. The greater the weight of soil in the bulk sample, the smaller are the standard errors of the estimated cyst populations per gramme, and the greater is the advantage gained by making up the sample from numerous points.

TABLE 2

*Estimated Standard Errors of the Cyst Populations per Gramme in Various Bulk Soil Samples Taken from a Field with 1·0 Cysts per Gramme**

Number of Sample Points†	Total Weight of Bulk Sample in Grammes (N)					
(s)	*500*	*1,000*	*2,500*	*5,000*	*10,000*	*Very Large*
	±	±	±	±	±	±
10 ..	·12	·10	·09	·09	·09	·09
25 ..	·09	·08	·06	·06	·06	·06
50 ..	·09	·07	·05	·05	·04	·04
100 ..	·08	·06	·04	·04	·03	·03
Very many	·08	·05	·03	·02	·02	—

* Standard errors for fields with other population densities may be estimated from the table by multiplying by the square root of the cyst population per gramme.

† Approximately $\frac{N}{s}$ grammes sampled from each station.

Thus, if only a low level of accuracy is required and a bulk sample of 500 g soil is taken, there is little advantage in making this up from more than 25 points.

However, if a more accurate estimate is needed and a bulk sample of some 2,500–5,000 g soil is taken, it will be worthwhile obtaining the soil from 50–100 points, particularly as Table 2 is based on average variance estimates and some fields are more heterogeneous than others. Unless great accuracy is required and the soil is sampled from very many points, there is little advantage in taking more than 5,000–10,000 g of soil in the bulk sample; indeed it may be a disadvantage to take much more soil because of the difficulty of thoroughly mixing a large sample.

It will only be worth making an effort to obtain a bulk sample which accurately represents the cyst population of the field, if the errors introduced in the laboratory sub-sampling and counting are not too great. This is illustrated in Table 3, which shows that if the laboratory count is made on only 50 g from a thoroughly mixed bulk sample, there is little advantage in taking more than a total of 500 g of soil from ten points in the field (or an equivalent field sample).

TABLE 3

*Estimated Standard Errors of Cyst Populations per Gramme Estimated by Various Sampling Procedures for a Field with 1·0 Cysts per Gramme**

Size of Field Sample (*Total Weight*)	Weight of Soil Examined in the Laboratory (*grammes*)			
	50	*100*	*200*	*400*
	±	±	±	±
500 g taken from 10 random points ..	·26	·20	·16	·14
1,000 g taken from 25 random points ..	·25	·19	·14	·11
2,500 g taken from 50 random points ..	·25	·18	·13	·10
Large sample from many random points ..	·24	·17	·12	·08

* Standard errors for fields with other population densities may be estimated from the table by multiplying by the square root of the cyst population per gramme.

However, if an accurate estimate of cyst population density is required, and the laboratory count is made on a 400 g sub-sample, the accuracy of the estimate will be considerably increased by taking some trouble in collecting the bulk sample; under these circumstances a total of at least 2,500 g of soil should be collected from fifty or more points in the field.

In Table 4 the approximate 5 per cent limits of actual cyst counts are given for different population densities and for different sizes of laboratory sample

TABLE 4

Approximate 5 per cent Limits for Actual Cyst Counts on Laboratory Samples of Various Sizes

(For Bulk Sample of 2,500 g from 50 Points or Equivalent)

Field Population (*Cysts per gramme*)	Weight Examined (*grammes*)		
	100	*200*	*400*
0·05	0–16	2–25	6–42
0·10	2–24	7–40	18–70
0·20	7–39	20–67	48–120
0·50	28–78	66–141	147–261
1·00	68–134	151–256	323–485

(taken from a bulk sample of 2,500 g from fifty points, or equivalent). From this table it would appear that, for many advisory purposes, laboratory examination of 100–200 g of soil is sufficient, provided the population level is 0·5 cysts g or more, but that it is desirable to examine rather more soil for fields with a population density around 0·1 cysts/g.

Thus, laboratory examination of 200 g of soil will almost certainly classify a field with 0·2 cysts/g or more as having at least 0·1 cysts/g; however, with a sample of this size there will still be an appreciable chance of estimating that a field with only 0·05 cysts/g has more than 0·1 cysts/g, or that a field with 0·1 cysts/g has less than 0·05 cysts/g. When this degree of error would be serious, it will be advisable to examine a further 200 g sub-sample if the count on the first 200 g is between 7 and 25 cysts.

STATISTICAL NOTE

Tables 2–4 in the previous sections were prepared on the following assumptions:

(1) That the square roots of cyst counts are approximately normally distributed with variances independent of the mean count for samples of a given type.
(2) That the variance of an actual count, x, is approximately $4x$ times the variance of the square root of that count.
(3) That the variance between counts on contiguous 50 g soil samples will be less than the estimated variance between counts for samples one yard apart, and probably greater than the estimated laboratory sampling variance. The smaller value has been assumed in the above calculations as this errs on the safe side by tending to over-emphasize the importance of having a well-distributed field sample.

Using the mean squares from the analyses given in the text, these assumptions lead to the following variance estimates, from which Tables 2–4 are readily derived:

$$\text{Variance of actual count} = mn^2 \left(\frac{2{\cdot}88}{N} + \frac{{\cdot}0768}{s} + \frac{2{\cdot}88}{n} \right)$$

$$\text{Variance of estimated cysts per gramme} = m \left(\frac{2{\cdot}88}{N} + \frac{{\cdot}0768}{s} + \frac{2{\cdot}88}{n} \right)$$

$$\text{Variance of square root of actual count} = \frac{n}{4} \left(\frac{2{\cdot}88}{N} + \frac{{\cdot}0768}{s} + \frac{2{\cdot}88}{n} \right)$$

where N = total weight of bulk sample from field (grammes); s = number of independent sampling points in the field; and n = weight of soil examined in the laboratory from the thoroughly mixed bulk sample (grammes).

SUMMARY

On the evidence of a small number of fields, it has been possible to give only an approximate indication of the expected accuracy of cyst population estimates obtained from samples of different sizes.

For routine estimation of cyst population, laboratory examination of 200 g soil, taken from a well mixed bulk sample of 2,500–5,000 g obtained from fifty or so randomly located points in a field, should usually prove sufficient. The accuracy to be expected from samples of this size has been indicated in Tables 3 and 4. When cyst populations are estimated to be in the region of 0·05–0·10 cysts/g (counts of 7–25 cysts per 200 g) a further 200 g sub-sample should be examined, if it is considered important to classify the population with some confidence as more or less than 0·1 cysts/g.

Although there are variable losses of cysts when using the Fenwick apparatus for recovering *H. major* from the soil (up to an average of 20 per cent for one of the fields studied), the average loss at all centres was only about 6 per cent of all cysts, and a rather smaller proportion of viable cysts was lost. It may therefore be concluded that the recovery of *H. major* from soil, employing the Fenwick apparatus with the techniques at present in use, is satisfactory for most

purposes. Although there were consistent differences between the cyst population estimates obtained at the different centres, the data discussed above do not indicate that the differences in detail of laboratory technique are of great importance, in the sense that the procedures adopted at one or more centres are definitely superior to the others. However, to ensure that estimates of cyst population density are comparable, the study group recommended that the following procedures should be adopted as far as this is practicable:

1. The dried soil should be mixed thoroughly by coning and quartering.
2. The Fenwick can should be filled with water before starting.
3. The metal from which the can is constructed does not appear to be important, but the interior finish should be smooth and free from cracks, ridges of solder, etc.
4. The mesh of the top sieve should be 16–20 to the inch.
5. The mesh of the collecting sieve should be 80–100 to the inch.
6. If the amount of floating debris makes a second flotation desirable, the first float should be thoroughly dried before refloating (see Hesling, 1956).
7. Cysts should be collected by filtration with bolting silk or filter paper, and not from the surface of a bowl or developer dish.

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COLOUR-BANDING OF CEREAL SEEDLINGS

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FIELD observations suggested that the colour-banding, or "rugby-stocking", symptoms sometimes seen in cereal seedlings at emergence might be associated with deep sowing. A small experiment was carried out at Anstey Hall, Cambridge in the winter and spring of 1952–53 to test this point. Nord Desprez wheat was sown on December 21 at depths ranging from 1 to 6 inches. Each sowing depth was randomized in rod-row plots in each of three blocks, and one half of each block was consolidated by trampling after the seed was sown.

Emergence of the first seedlings was noted on February 12, and by the following week all the plots showed a number of emerging shoots, some of which were yellow and the remainder green. Colour-banding was visible on the yellow shoots within two days of emergence. The affected seedlings developed horizontal green bands varying in number from one to six (Plate II). The banding

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was usually complete by the sixth day after emergence. The yellow bands turned orange or red, often surrounding a white central area. Counts of the mean numbers of plants per 3 feet of drill, and of the percentages showing colour-banding, at two and at four days after emergence, for each depth of sowing are given in the Table.

Colour-banding in Relation to Depth of Sowing

Depth of Sowing	Two Days after Emergence				Four Days after Emergence			
	Trampled after Sowing		Not Trampled		Trampled after Sowing		Not Trampled	
inches	*No. of Plants*	*Per cent Banded*	*No. of Plants*	*Per cent Banded*	*No. of Plants*	*Per cent Banded*	*No. of Plants*	*Per cent Banded*
Nord Desprez, emerged February 12								
1	91	0	103	0·6	95	0·7	105	1·0
2	93	0·5	100	0·4	95	1·0	101	0·3
3	90	3·0	98	1·9	98	4·6	103	1·3
4	72	8·1	96	1·9	87	6·1	97	3·5
5	74	13·5	89	14·1	89	16·3	100	10·5
6	29	38·5	29	21·5	55	42·3	46	26·1
Atle, emerged March 1								
1	57	0	61	0	61	0	66	0
2	55	0	56	0	61	0	63	0
3	57	1·1	58	0·5	60	1·0	64	0·5
4	63	2·5	67	1·4	70	2·3	72	1·3
5	57	12·3	58	4·1	64	11·6	64	4·7
6	36	27·6	48	11·4	41	28·1	46	12·4

The banding effect on the Nord Desprez wheat began to disappear about eight days after the shoots had emerged above ground. The affected plants eventually became brownish-green in colour without any traces of the original banding, and their future development did not appear to be affected.

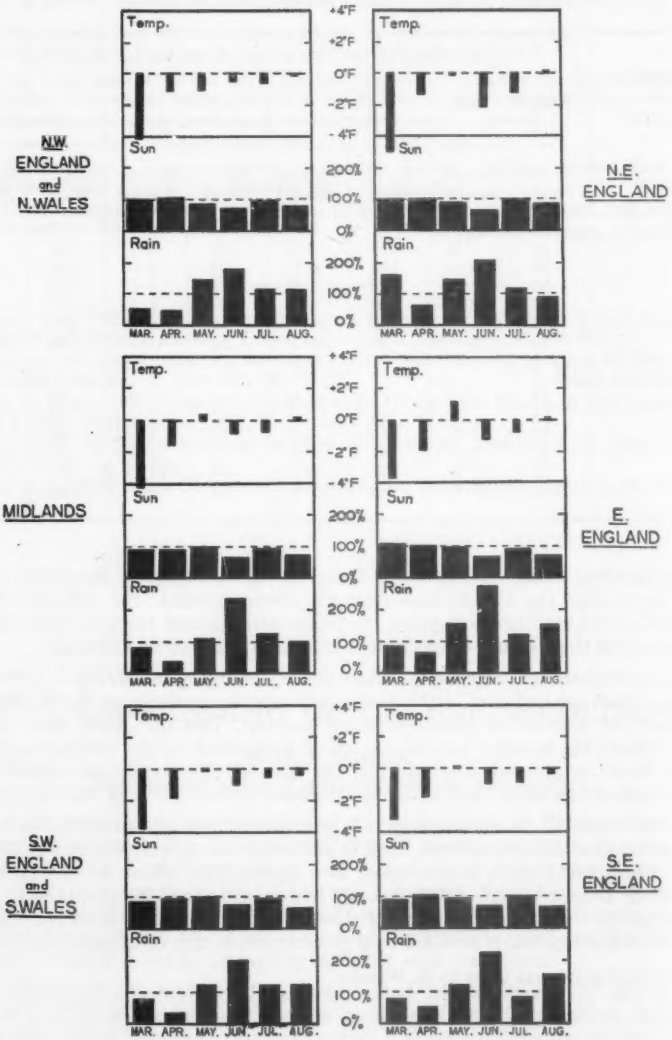
The experiment was repeated on Atle wheat, emerging on March 3, 1953. The results, which are included in the Table, were similar to those on Nord Desprez, although the amount of colour-banding was less. This may have been due to differences in the weather conditions, which were much colder during the period of the first experiment than during the second. In glasshouse experiments carried out at the same time as those outdoors, colour-banding did not appear.

Further cases of colour-banding in winter wheat and also in winter oats have been seen since the experiments, and in each case the sowing depth was at least four inches. Field observations have also shown that where consolidation of the soil by tractor wheels before sowing had taken place, the plants were more or less normal. In the experiments at Cambridge, consolidation of the soil was done after sowing and it resulted in a greater percentage of affected plants.

The photograph was taken by H. Wheeler.

MARCH-AUGUST WEATHER 1958

Variations from Average



THE WEATHER OF ENGLAND AND WALES*

SPRING AND SUMMER 1958

FOLLOWING a winter that was relatively mild in the southern parts of England, March was a very cold month everywhere, with mean temperatures some 4 degrees below average. Snow covered the uplands of Scotland for much of the month and north-east England was under snow from the 8th to the 16th; only occasional spells of snow showers affected the southern counties. The first two weeks of April were similarly colder than average, but the latter part of the month was much milder. Snow showers were rather common during the cold spell but total amounts of precipitation were small, and April ranked as the only dry month of the summer half-year. May brought changeable weather with frequent spells of rain and average temperatures; there was almost a total absence of air frosts.

June was a very wet month indeed; the rainfall over England and Wales was more than twice average and the wettest since 1912. It was also lacking in sunshine and slightly below average in temperature. July was less wet but similar in character, thunder being especially frequent. There was a very short warm spell about the 8th, with day maxima above 80°F. August was another wet, thundery month with average temperatures; the days were cool but the nights were relatively mild and diurnal ranges of temperature were smaller than usual. As a result, average relative humidities were high not only during the night but also during daylight hours. A few limited areas had less than average rainfall, but most places were deficient in sunshine.

The weather for each of the six months, March to August (inclusive), is represented graphically on page 36. Figures for each of the six districts of England and Wales are shown in terms of the deviation from average of air temperature, sunshine and rainfall. The outstanding features are the very cold March, the dull and wet summer and the near average mean temperatures during the last four months. This last feature conceals the tendency to above average night minima and below average day maxima, which was more conducive to high humidities than the mere occurrence of frequent rain.

NEW OR UNCOMMON PLANT PESTS

Stem and Bulb Eelworm Attacking Swedes. *Ditylenchus dipsaci* (Kühn) Filipjev is not recorded as attacking swedes, *Brassica napus* L. v. *Napobrassica*, in the 1956 edition of Goodey and Franklin's *The Nematode Parasites of Plants Catalogued under their Hosts*, although Newton and Duthoit (*Plant Pathology*, 1954, **3**, 140), refer to distortion and lesions in a swede crop growing next to infested potatoes. In May 1958, my attention was called to a three-acre patch in a twelve-acre crop of green top swedes at Washington, Co. Durham. At the singling stage the affected plants showed severe malformation of the cotyledons and largest leaves, the petioles were thickened, arched over backwards, and scarred, whilst the laminae were thick and brittle (Plate III, 1). In some plants the growing point was dead (Plate III, 2), death of the whole plants following, while in others there was bud proliferation at a very early growth stage suggesting the ultimate development of a "many-neck" condition. The petioles and laminae

* These notes and the diagram on page 36 are published by permission of the Director General of the Meteorological Office.

of such plants contained large numbers of eelworm adults, larvae, and eggs, identified as *D. dipsaci* and subsequently confirmed by J. B. Goodey. The field was cropped with wheat in 1957, potatoes in 1956, and wheat in 1955. Prior to this the field had almost certainly been cropped heavily with oats. The principal weeds present were: *Carduus arvensis* L, *Sonchus arvensis* L, *Senecio vulgaris* L, *Capsella bursa-pastoris* (L.) Medic., *Cerastium* spp, and self-set potatoes. Most of these have been recorded as hosts of stem and bulb eelworm, but despite extensive laboratory examination none from the field was found to be infested. Tests were also done using inoculum from the swedes and oat seedlings raised in sterilized loam. Up to six weeks after inoculation only one or two eelworms were found in the oat plants and breeding did not take place. Complementary transfers, using material from oats showing "tulip root" symptoms, to swedes growing in sterilized loam were negative. It appears that the strain affecting swedes differs from the one affecting oats.

J. H. WHITE

White Cysts of Cereal Root Eelworm on Oat Stubble. On an advisory visit to a field of oat stubble in February 1958 it was found that white cysts were present on the roots of the old plants. The field was on light sandy soil near Feltwell, Norfolk, and was known to be heavily infested with *Heterodera major* (O. Schmidt) which had caused patchiness in the 1957 crop. The oats had been undersown with sainfoin and a few weed grasses were also present. Soil samples were taken and examined whilst still damp: ten 100 g samples of soil and plant debris were washed through a Fenwick can, the sludge being put through an elutriator. From these samples 245 cysts were recovered, thirteen of them being white; some of the white cysts were immature, but others were nearing maturity and contained eggs. As the soil dried, samples were examined periodically, but no more white cysts were found, possibly because the more immature ones had died and the mature ones had turned brown; several of the latter were pale brown in colour, and all of the cysts recovered by elutriation appeared to be of recent origin, many having the sub-crystalline layer still attached. From these observations it is thought that, on unploughed fields, development of cereal root eelworm may continue during mild winters.

L. J. COPPOCK and A. L. WINFIELD

APHIDS ON CEREALS AND GRASSES IN 1957

During April 1957 severe aphid attacks were reported on cereals (chiefly wheat and oats) and grass seed crops (cocksfoot, fescues, ryegrass and timothy). The attacks were the heaviest since the spring of 1949, which also followed a mild winter and early spring. Species most frequently reported were *Metopolophium festucae* (Theob.) and *Rhopalosiphum padi* (L.), the latter being the more serious and frequently associated with patches of yellowing and dead plants. Widespread infestations were reported by the advisory entomologists in Lancs, West Riding of Yorks, Cheshire, Staffs, Lincs: Holland, Kesteven and Lindsey, Bedford, Cambridge, Essex, Suffolk, Kent, West Sussex, Middlesex, Oxford, I. of Wight, Devon, Cardigan and Pembroke. Attacks declined rapidly late in May when heavy parasitism was recorded. There is a difference of opinion about the relationship between aphid attack and leaf discoloration, probably because other factors such as drought and virus diseases (for example, barley yellow dwarf virus on oats) can contribute to it. Nevertheless it seems clear that a considerable degree of yellow flecking and streaking and purple spotting, followed by more pronounced general yellowing and death of the leaves, can be attributed to aphid attack alone.

H. W. JANSON

(75345) Wt. 25 K.9 2/59 Hw.